Specification Amendments

Please amend the specification on page 1 by inserting the following heading and paragraph before the "Field of the Invention."

CROSS-REFERENCE

This application is a national stage application of PCT patent application number PCT/US2003/025058 filed August 11, 2003, and claims priority from US Provisional patent application No. 60/403,330 filed August 12, 2002, each of which are incorporated herein in their entirety.

Please replace the paragraph beginning on page 9 at line 14 with the following paragraph (marked with underlining to denote added text):

The DNA sequence of the complete E. coli K-12 appA gene was originally reported by Dassa et al. (1990). Since that original report, however, a number of appA gene variants (naturally occurring or laboratory generated) have been described. Ostanin et al. (1992) used site-directed mutagenesis to examine the catalytic importance of 2 histidine and 4 arginine residues which are conserved in a number of acid phosphatases. The replacement of Arg16 (R16A) or His17 (H17N) within the conserved N-terminal RHGXRXP (SEQ ID No. 30) motif of the E. coli AppA protein completely abolished activity on p-nitrophenyl phosphate (pNPP). Mutagenesis of Arg20 (R20A), Arg92 (R92A) and His303 (H303A) resulted in proteins with only 0.4% the activity of the wild-type (WT) enzyme while replacement of Arg63 (R63A) did not affect activity. Site directed mutagenesis experiments, designed to explore the role of Asp304 as a proton donor, demonstrated only small decreases in Km values of the substrate pNPP for the mutants D304A and D304Q (Ostanin et al., 1993). However, Vmax values were greatly reduced.

Please replace the paragraph beginning on page 14 at line 11 with the following paragraph (marked with underlining to denote added text).

FIG. 12 show the amino acid sequence of the 18B2 control AppA phytase and locations of variations in the sequence found in different mutants of the invention. The composite Bacillus signal sequence (residues 1-30) is underlined and the conserved RHGXRXP (SEQ ID No. 30) motif characteristic for histidine phosphatases is in bold italics. Only differences from the control sequence are indicated for the AppA sequences of the identified mutants. Each of the mutant amino acid sequences comprises a substitution at residue 143 (corresponding to residue 113 of the mature E. coli K-12 AppA phytase). Bacillus subtilis hosts expressing a mutant AppA shown in this figure had improved phytase activity as compared with clones expressing the control AppA.

Please replace the paragraph beginning on page 17 at line 19 with the following paragraph (marked with underlining to denote added text).

The term "amino acid residue equivalent to", "amino acid corresponding to" and grammatical equivalents thereof is used herein to refer to a amino acid residue of a protein having the similar position and effect as that indicated in a particular amino acid sequence of a particular protein. For example, the residue of an AppA protein equivalent to amino acid 46 of the EBC18B2 AppA protein of FIG. 12 is a residue equivalent to the first Arginine of the conserved RHGXRXP (SEQ ID No. 30) motif characteristic of histide phosphatases such as the E. coli AppA phytase. The amino acid sequence and crystal structure of many phytases are known (see e.g., Lim et al., 2000; Pandey et al., 2001; Kerovuo et al., 2000; and Kostrewa et al., 1997). The person of skill in the art will recognize the equivalence of specified residues in comparable phytase proteins.

Please replace the paragraph beginning on page 74 at line 25 and ending on page 75 line 1 with the following paragraph (marked with underlining to denote added text).

The appA variants PHY850 and PHY902 were identified in the third generation error prone PCR libraries #4A and #5A, respectively, (see, FIG. 4). However, sequence analysis of clones PHY735 and PHY736 demonstrates that the H143R mutation could be detected as early as the second generation error prone PCR library #2 (FIG. 12). PHY736 was found to harbor two additional amino acid changes (W89R and A103V). Two H143R containing mutants, PHY679 and PHY846, were identified as hits from the third generation libraries #3A and #4A, respectively. PHY679 contains three additional amino acid changes (T56A, N156K and G258P). The T56A change of PHY679 is located 4 residues from the AppA active site RHGXRXP (SEQ ID No. 30) motif Clone PHY846 contains only one additional change, Q214R.

Please replace the paragraph beginning on page 56 at line 14 and ending at page 56, line 20 with the following (marked with underlining to denote added text).

The following DNA primers have been constructed for use in amplification of phytase genes from the libraries constructed from the various microorganisms.
*AppA*3F 5'-atgaaagcgatcttaat (SEQ ID No. 12)
*AppA*5F 5'-cgtcatggtgtgcgtgctcc (SEQ ID No. 13)
*AppA*6F 5'-cgccagaggttgcccg (SEQ ID No. 14)
AppA 7R 5'-gcggctggcaacctctgg (SEQ ID No. 15)
*AppA*4R 5'-ttacaaactgcacgccggtatgcgtgcgtgcttcatt (SEQ ID No. 16)